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Evidence that brain prostaglandin E_2 is involved in physiological sleep–wake regulation in rats

(antagonist/AH 6809/intraventricular infusion/brain temperature)

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ABSTRACT **We reported in previous studies that prostaglandin E_2 (PGE_2) has central effects of augmenting wakefulness and suppressing slow-wave sleep (SWS) and paradoxical sleep (PS) in rats. In the present study, we tested the effect of AH 6809, an antagonist of PGE_2 receptors, on sleep–wake activities. AH 6809 in saline was infused continuously into the third ventricle of freely moving rats at a rate of 2.1, 6.3, and 21 pmol/min from 2300 to 0500 hr. During the infusion at 21 pmol/min, wakefulness decreased to 82%, and SWS and PS increased to 122% and 161%, of the respective baseline values. These changes can be explained by AH 6809 antagonizing the endogenous PGE_2 that acts to augment wakefulness in the brain. This explanation is supported by the fact that the infusion of AH 6809 at 21 pmol/min inhibited the wakefulness-promoting effect of PGE_2 infused at 10 pmol/min. Moreover, the PGE_2-related mechanisms for regulating sleep–wake activities may be different from those producing hyperthermia, because AH 6809 at 21 pmol/min had no primary effect on brain temperature and did not antagonize the hyperthermia produced by the PGE_2 infusion. A diurnal infusion (1200 to 1800 hr) of AH 6809 at 21 pmol/min produced similar effects on sleep–wake activities compared with the nocturnal infusion (2300 to 0500 hr), although the PS increase was not significant, suggesting that the PGE_2-related mechanisms are acting all day long with or without a circadian rhythm. These findings strongly suggest that endogenous PGE_2 in the brain is involved in the physiological mechanisms for regulating sleep–wake activities.**

We demonstrated recently that prostaglandin E_2 (PGE_2) has central effects of promoting wakefulness and suppressing both slow-wave sleep (SWS) and paradoxical sleep (PS) (1, 2). This effect of PGE_2 on sleep–wake activities was confirmed by a study in which continuous infusion was carried out with freely moving rats exhibiting a normal circadian rhythm (2). Moreover, the minimum effective doses of PGE_2 in our previous studies were low enough to allow us to speculate that endogenous PGE_2 in the brain is involved in the physiological sleep–wake regulation.

To examine this issue further, we tested the effect of a prostaglandin antagonist, AH 6809, on sleep–wake activities. If AH 6809, which has been shown to be an antagonist of PGE_2 receptors (3), inhibits the awaking effect of PGE_2, and if endogenous PGE_2 in the brain is involved in the physiological sleep–wake regulation, then administration of AH 6809 into the brain should decrease wakefulness and promote sleep.

It is widely accepted that sleep–wake activities are closely related to body and brain temperatures. Since PGE_2 produces hyperthermia (4–7), it has been a controversial issue as to whether the effect of PGE_2 on sleep–wake activities results from the temperature changes caused by PGE_2.

In this study, the effect of AH 6809 on sleep–wake activities was examined along with its effect on the brain temperature in freely moving rats with the continuous infusion system used in our previous study (2).

MATERIALS AND METHODS

Male Sprague–Dawley rats (280–380 g) that were raised in our closed colony were subjected to surgical operation under pentobarbital anesthesia (50 mg per kg of body weight). A stainless steel cannula (o.d., 0.35 mm) for continuous infusion into the third ventricle was stereotaxically placed 3.4 mm lateral from the bregma and inserted 9 mm from the surface of the cortex at an angle of 20° from the midsagittal plane. Electrodes for electroencephalogram recordings were attached onto the frontoparietal cortex and an indifferent electrode was placed on the frontal skull. Some rats were implanted with a thermistor probe with the tip in the thalamus. The cannula, electrodes, and thermistor probe were fixed to the skull with dental acrylic resin. Electrodes for electromyogram recordings were hooked in the neck muscles. Details have been described (2, 8, 9).

After surgery, the rats were permitted to recover in individual cages for ≈1 week. Then, saline infusion into the third ventricle was initiated in each rat, continuing until the end of the experiment except when a test solution was infused. The infusion of saline or test substance(s) dissolved in saline was carried out at a rate of 10 μl/hr. The infusion and the recordings of electroencephalogram, electromyogram, and brain temperature were carried out via a slip ring, designed so that the behavioral movement of the rat was not restricted. Rats were habituated to the continuous infusion and other experimental conditions for ≈5 days before the beginning of the experiment. A typical experiment consisted of 3 consecutive recording days—i.e., baseline day, experimental day, and recovery day. The experimentation was conducted in a sound-proof and electromagnetically shielded room maintained at 25°C ± 1°C and 60% ± 6% relative humidity with a 12-hr light (0800 to 2000 hr)/12-hr dark (2000 to 0800 hr) cycle.

From 2300 to 0500 hr or from 1200 to 1800 hr on the experimental day, one of the following infusions was administered instead of the saline infusion: (*i*) AH 6809 in saline at

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Abbreviations: SWS, slow-wave sleep; PS, paradoxical sleep; PGE_2, prostaglandin E_2.

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2.1, 6.3, or 21 pmol/min (2300 to 0500 hr); (*ii*) PGE_2 in saline at 10 pmol/min (2300 to 0500 hr); (*iii*) AH 6809 and PGE_2 dissolved together in saline at 21 and 10 pmol/min, respectively (2300 to 0500 hr); and (*iv*) AH 6809 given diurnally at 21 pmol/min (1200 to 1800 hr). We could not adopt higher rates in the infusion of AH 6809 because of its limited solubility in saline.

The electroencephalogram and electromyogram recordings were scored as SWS, PS, or wakefulness according to the criteria described by Honda *et al.* (8). The brain temperature was recorded by means of a thermistor probe (Takara, Yokohama, Japan) at 3-min intervals. The mean value of recordings in each 30-min period was defined as the temperature of that period. Data of the experimental and recovery days were compared with data of the baseline day by the paired *t* test.

PGE_2 and AH 6809 were kindly provided by Ono Pharmaceuticals (Osaka, Japan) and Glaxo, respectively.

RESULTS

AH 6809 was continuously infused from 2300 to 0500 hr at rates of 2.1, 6.3, or 21 pmol/min. At the highest rate, SWS and PS increased to 122% and 161%, and wakefulness decreased to 82%, of the respective baseline values (Fig. 1). At this rate, PS increased from the first hour of the infusion and remained above the baseline amount until 1 hr after cessation of the infusion (Fig. 2). SWS similarly increased during the infusion of AH 6809. In regard to the episodes of SWS, PS, and wakefulness, AH 6809 produced no significant changes in their duration and number, except that the PS episode was significantly prolonged (Table 1). The increases in SWS and PS by AH 6809 resulted from the synergism of prolongation and more frequent occurrence of episodes. The decrease in time spent in wakefulness was mainly due to the shortened duration of episodes. After 1200 hr in the following

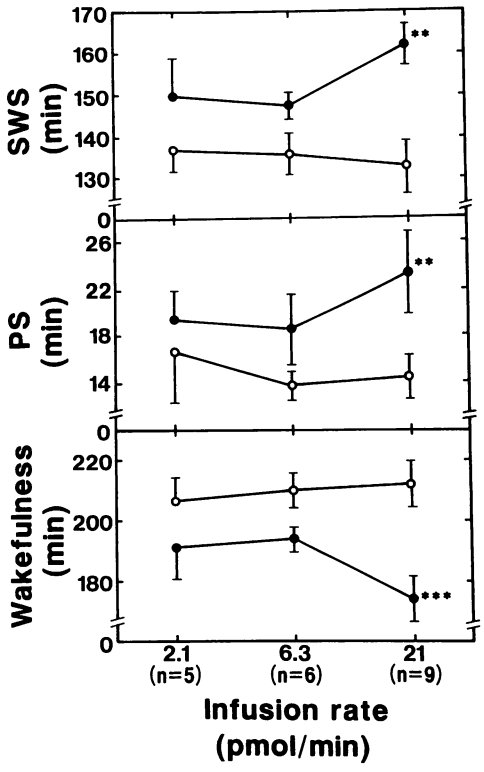

FIG. 1. Time (min) spent in SWS, PS, and wakefulness during nocturnal infusion (2300 to 0500 hr) of AH 6809. ○ and ●, mean sleep or wakefulness (±SEM) of the baseline and experiment, respectively. $**P < 0.01$, $***P < 0.001$ by paired *t* test.

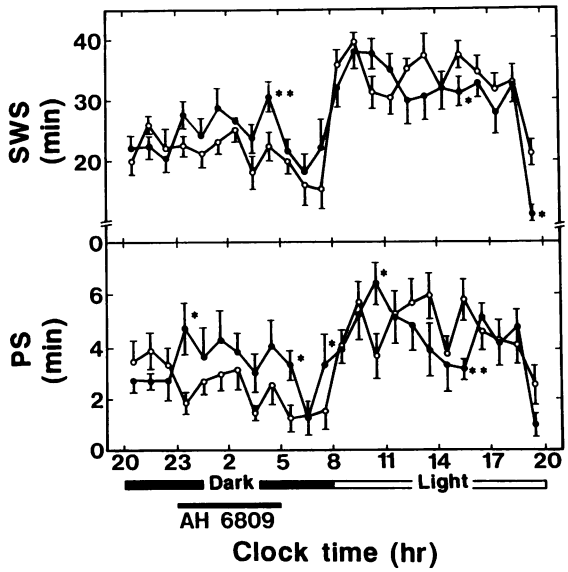

FIG. 2. Changes in hourly SWS and PS before, during, and after infusion of AH 6809 at 21 pmol/min. AH 6809 in saline was infused on the experimental day from 2300 to 0500 hr (horizontal bar) into the third ventricle of freely moving rats ($n = 9$) that were otherwise infused with saline solution. The environmental light and dark periods were 0800 to 2000 hr and 2000 to 0800 hr, respectively. ○ and ●, hourly mean SWS or PS (±SEM) of the baseline and the experimental days, respectively. $*P < 0.05$, $**P < 0.01$ by paired *t* test.

light period, SWS and PS tended to decrease (Fig. 2), which may be interpreted as a rebound phenomenon. Dissociation from the baseline of the hourly amounts of SWS, PS, and wakefulness disappeared on the recovery day.

A solution of PGE_2 or PGE_2 and AH 6809 dissolved together in saline was infused from 2300 to 0500 hr (Table 1). During the PGE_2 infusion of 10 pmol/min, SWS and PS decreased to 82% and 39%, respectively, and wakefulness increased to 118%, of the respective baseline values. The SWS reduction was due to shortening of episode duration, and the PS reduction was due to both the shortening and less frequent occurrence of episodes, while the increase in wakefulness resulted from an increase in the number of episodes.

With the infusion of PGE_2 (10 pmol/min) and AH 6809 (21 pmol/min) dissolved together in saline, no changes occurred in the total time of SWS, PS, and wakefulness (Table 1).

Concerning brain temperature, the infusion of AH 6809 produced no changes from the baseline (Fig. 3*A'*). After the infusion, a slight temperature elevation was observed from 1230 hr onward during the following light period. This elevation of temperature occurred simultaneously with decreases in SWS and PS (Fig. 2) and an increase in wakefulness (Fig. 3*A*).

With the infusion of PGE_2 (10 pmol/min), the brain temperature increased sharply, concomitantly with a reduction in SWS and PS and an augmentation in wakefulness (Fig. 3 *C* and *C'*). With the infusion of AH 6809 (21 pmol/min) and PGE_2 (10 pmol/min) dissolved together in saline, the brain temperature increased (Fig. 3*B'*), and the magnitude of the increase was not attenuated when compared with the temperature elevation caused by the infusion of PGE_2. The infusion produced no changes in the hourly amount of wakefulness (Fig. 3*B*).

During the diurnal infusion (1200 to 1800 hr) of AH 6809, SWS increased and wakefulness decreased (Table 2). PS tended to increase, but the change was not statistically significant. The increase in the total time of SWS was caused by the prolonged duration of episodes. The decreased wakefulness resulted from synergism of the shortened duration

Table 1. Changes in sleep–wake activities during nocturnal infusion of AH 6809 and/or PGE_2

Treatment	*n*	Activity	Total time, min			Episode, % of baseline	
			Baseline (B)	Experiment (E)	E/B, %	Duration	Number
AH 6809 (21 pmol/min)	9	SWS	133.0 ± 6.4	162.2 ± 5.0*	122	111.1 ± 5.8	114.3 ± 9.6
		PS	14.6 ± 1.9	23.6 ± 3.6*	161	122.7 ± 9.0†	142.2 ± 18.9
		Wakefulness	212.2 ± 7.7	174.1 ± 7.6‡	82	86.4 ± 8.5	101.9 ± 9.3
AH 6809 (21 pmol/min) + PGE_2 (10 pmol/min)	6	SWS	134.9 ± 7.6	146.8 ± 10.4	109	96.4 ± 9.9	119.4 ± 12.3
		PS	19.0 ± 1.3	16.6 ± 4.6	87	77.1 ± 4.8†	118.5 ± 32.6
		Wakefulness	205.9 ± 7.9	196.4 ± 13.3	95	93.3 ± 20.8	114.4 ± 11.3
PGE_2 (10 pmol/min)	5	SWS	147.9 ± 5.7	121.6 ± 11.3†	82	59.2 ± 1.9‡	139.4 ± 12.2†
		PS	16.2 ± 1.1	6.3 ± 2.0*	39	87.9 ± 4.2†	42.5 ± 12.5*
		Wakefulness	195.8 ± 6.7	232.0 ± 10.4*	118	86.8 ± 10.5	142.7 ± 13.1†

AH 6809, AH 6809 together with PGE_2, or PGE_2 dissolved in saline was infused into the third ventricle of freely moving rats from 2300 to 0500 hr. Total time, episode durations, and episode numbers of SWS, PS, and wakefulness during this period were compared with those under the saline infusion during the same period on the baseline day. Each value represents mean ± SEM. Paired *t* test was used for the statistical analyses.
*$P < 0.01$.
†$P < 0.05$.
‡$P < 0.001$.

and less frequent occurrence of episodes. The apparent increase in the number of PS episodes produced the increasing tendency in the total time of PS.

DISCUSSION

In this study, a continuous infusion of AH 6809 and/or PGE_2 in saline or saline solution was carried out at such a low rate (10 μl/hr) so as not to produce untoward responses. The behavioral movement of the rats was not restricted due to the aid of slip rings. Under these experimental conditions, the rats behaved normally and exhibited a clear circadian rhythm on the baseline day, as described in detail (2).

AH 6809 reportedly antagonizes PGE_2 on isolated smooth muscle preparations, and it is classified as an antagonist of EP_1 receptors, a subtype of PGE_2 receptors (3). In the case of human platelets, AH 6809 was shown to be a weak but specific blocking drug of PGD_2 receptors (10).

In this study, the continuous infusion of AH 6809 at 21 pmol/min reduced wakefulness and increased SWS and PS. PGE_2 and PGD_2 are known to have opposite effects on sleep–wake activities; PGE_2 augments wakefulness (1, 2, 11) while PGD_2 promotes sleep (11–14). Therefore, the result obtained by the infusion of AH 6809 in this study is explained by AH 6809 antagonizing endogenous PGE_2 that promotes wakefulness in the brain. This explanation is supported by the fact that an infusion of AH 6809 (21 pmol/min) and PGE_2 (10 pmol/min) dissolved together in saline negated the changes in sleep–wake activities produced by the infusion of PGE_2 at 10 pmol/min.

We demonstrated in our previous studies that PGE_2 reduces sleep without any specificity on SWS or PS (1, 2). The

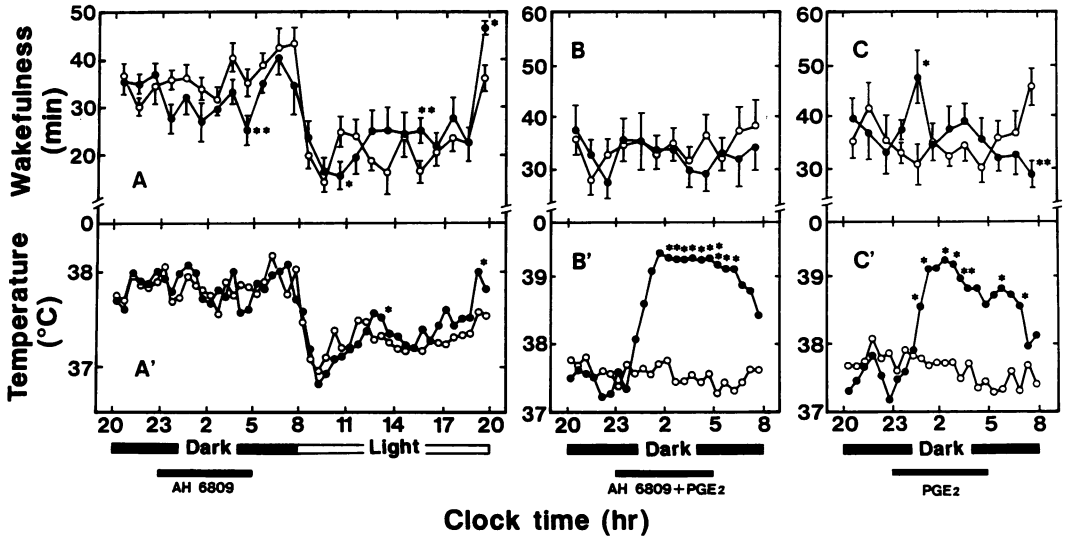

FIG. 3. Changes in wakefulness and brain temperature before, during, and after infusion of AH 6809 (*A* and *A'*), AH 6809 with PGE_2 (*B* and *B'*), or PGE_2 (*C* and *C'*). A solution of AH 6809 and/or PGE_2 in saline was infused from 2300 to 0500 hr (horizontal bars) into the third ventricle of freely moving rats that were otherwise infused with saline solution. The environmental light and dark periods were 0800 to 2000 hr and 2000 to 0800 hr, respectively. The infusion rates of AH 6809 and PGE_2 were 21 and 10 pmol/min, respectively. (*Upper*) Hourly changes in wakefulness (*A*, $n = 9$; *B*, $n = 6$; *C*, $n = 5$). With some rats in these groups, the brain temperature was simultaneously recorded at 3-min intervals. In each rat, the recordings of every 30 min were averaged, being defined as the temperature value for that period. (*Lower*) Each point represents the mean of the temperature values obtained by this procedure (*A'*, $n = 5$; *B'*, $n = 3$; *C'*, $n = 3$). Vertical bars representing SEM are omitted to avoid complication. SEM values in *A'*, *B'*, and *C'* were within 0.42°C, 0.48°C, and 0.81°C, respectively. ○ and ●, baseline and experimental values, respectively. *$P < 0.05$, **$P < 0.01$ by paired *t* test.

Table 2. Changes in sleep–wake activities during diurnal infusion of AH 6809

Treatment	*n*	Activity	Total time, min			Episode, % of baseline	
			Baseline (B)	Experiment (E)	E/B, %	Duration	Number
AH 6809 (21 pmol/min)	7	SWS	225.4 ± 6.6	241.7 ± 6.5*	107	121.4 ± 8.8†	90.7 ± 5.5
		PS	31.1 ± 3.7	33.6 ± 2.1	108	98.0 ± 6.6	118.4 ± 10.7
		Wakefulness	103.4 ± 7.0	84.6 ± 7.9*	82	91.4 ± 9.3	92.4 ± 6.1

AH 6809 dissolved in saline was infused into the third ventricle of freely moving rats from 1200 to 1800 hr. Total time, episode durations, and episode numbers of SWS, PS, and wakefulness during this period were compared with those under the saline infusion during the same period on the baseline day. Each value represents mean ± SEM. Paired *t* test was used for the statistical analyses.
*$P < 0.01$.
†$P < 0.05$.

infusion of AH 6809 at 21 pmol/min in this study inhibited both the reductions of SWS and PS that were produced by PGE_2. The nocturnal infusion of AH 6809 alone increased both SWS and PS; no specificity on SWS or PS was observed.

The increases in SWS and PS during the nocturnal infusion of AH 6809 resulted from the synergism of prolongation and a more frequent occurrence of respective episodes, while the decrease in wakefulness by AH 6809 was caused by a shortened duration of wakefulness episodes. Rats under the infusion of AH 6809 slept well and did not stay awake for long. On the other hand, rats under the PGE_2 infusion did not sleep very long.

In the present study as well as in our previous studies, PGE_2 produced hyperthermia, which is consistent with other published results (4–7). Since sleep–wake activities are said to be closely related to the temperature changes of an animal, the question is raised as to whether the effect of PGE_2 on sleep–wake activities is of a primary and specific nature or is one that is caused by the hyperthermia produced by PGE_2. It has been repeatedly reported that a dissociation sometimes occurs between the response of sleep–wake activities and that of temperature (15–17). Moreover, injection of the minimum effective dose of PGE_2 into the brain produced sleep suppression without any change in temperature in our previous study (1). In this study, AH 6809 infusion at 21 pmol/min did not antagonize the hyperthermia produced by PGE_2 infusion at 10 pmol/min, although the former infusion inhibited the wakefulness augmentation by the latter infusion. The infusion of AH 6809 alone at 21 pmol/min promoted sleep without affecting temperature. These findings strongly suggest that PGE_2 produces a high propensity for the waking state through mechanisms different from those responsible for hyperthermia.

The PGE_2 infusion at night produced similar changes in sleep–wake activities when compared with the diurnal infusion that was carried out in our previous study (2). Furthermore, the diurnal infusion of AH 6809 produced similar changes to those produced by the nocturnal infusion of the antagonist, although the PS increase caused by the diurnal infusion was not significant. Therefore, PGE_2-related mechanisms in sleep–wake activities may be acting all day long, with or without a circadian rhythm.

The changes in sleep–wake activities caused by AH 6809 were followed by a rebound. We previously reported that a rebound occurs after sleep suppression produced by PGE_2 (2). It has been proposed that natural sleep–wake activities are partially controlled by a homeostatic process (18). In view of this proposition, the changes produced by PGE_2 and its antagonist comply with natural sleep–wake activities.

The results obtained in this study are in good agreement with the supposition that the endogenous PGE_2 of the brain plays a fundamental role in the physiological mechanism of regulating sleep–wake activities.

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